TESTIMONY OF

MICHAEL V. CALLAHAN, MD, DTM&H

DIRECTOR, BIOLOGICAL THREAT DEFENSE & MASS-CASUALTY CARE

CENTER FOR INTEGRATION OF MEDICINE & INNOVATIVE TECHNOLOGY (CIMIT)

MASSACHUSETTS GENERAL HOSPITAL/ HARVARD MEDICAL SCHOOL

TO THE

SUBCOMMITTEE ON PREVENTION OF NUCLEAR AND BIOLOGICAL ATTACK

UNITED STATES HOUSE OF REPRESENTATIVES
109TH CONGRESS

ENGINEERING BIOAGENTS: LESSONS FROM THE OFFENSIVE U.S. AND RUSSIAN PROGRAMS

13 JULY, 2005

NOT FOR PUBLICATION
UNTIL RELEASED BY
THE UNITED STATES HOUSE OF REPRESENTATIVES

Engineering Bio-agents: Lessons from the Offensive U.S. and Russian Programs

Mr. Chairman, distinguished Members, it is an honor to appear before you to present information on the threat of traditional and next-generation biological weapons. My perspective is derived from experiences as a tropical medicine physician who studies and treats the diseases caused by these agents, from experiences working with former biological weapon scientists in Russia, and threat assessment activities on behalf of the Department of Homeland Security's National Bioterrorism Analysis and Countermeasures Center (NBACC).

I am a staff physician in the Division of Infectious Diseases at Massachusetts General Hospital in Boston, Massachusetts, and the Director of Biological Threat Defense at the Center for Integration of Medicine and Innovative Technology (CIMIT). CIMIT is a multi-institution, non-profit research organization funded by the U.S. Government to identify near-term solutions for critical military and civilian medical problems. Since January 2002, I have also worked with the U.S. Department of State, in particular with the Bio-Industry Initiative (BII), a program which uses the U.S. biotechnology market and academic collaborations to redirect former Soviet biological weapons scientists to peaceful, sustainable medical research. Prior to this position I was on faculty at the Center for International Health at Boston University where I served as clinical investigator for tropical medicine research projects in sub-Saharan Africa. I currently maintain tropical disease research activities in five developing countries, which is pertinent to the discussion below. Since the October 2001 anthrax attack, I have worked with biological terrorism working groups from the National Academy of Science, the Department of Defense, and the Department of Homeland Security. My focus areas are risk analysis of small scale biological weapon production, and consequence management following mass-casualty infections and poisonings.

This subcommittee has asked that I provide some perspective on the threat of engineered biological weapons. As there is considerable debate about several aspects of biological weapons, I have attempted to support this testimony with photographs from the field and from laboratory modeling activities. I will emphasize here that I am not an expert on the former U.S. biological weapons program that was disbanded in 1971. I also understand that Dr. Alibek will provide testimony on the Soviet biological weapons program under Biopreparat. My reference to the FSU (Former Soviet Union) program will therefore, be restricted to information gained from ongoing research collaborations with ex-biological weapons scientists from 10 Russian institutes. It should be emphasized that my experiences helping BII to develop drug and vaccine commercialization opportunities for former weapons scientists have resulted in access to several institutions previously closed to westerners (Figure 1). Further transparency is gained, perhaps ironically, by relationships forged from my medical care of former weapons scientists and their family members, and on occasion, emergency medical consultation to infections resulting from laboratory accidents. Finally, it is probably relevant that my experiences conducting clinical research in remote African and Asian locales have sensitized me to some of the challenges a terrorist lab would encounter when attempting to make a biological weapon in an austere environment (Figure 2).

What is our current understanding of engineered biological weapons?

Most experts agree that biological weapons are the original weapons of mass destruction. Throughout history, the overwhelming majority of biological weapons were used in a crude form. For example the first recorded use of biological agents was in 1346 when the Tartars catapulted plague-ridden corpses into the city of Kafka. In more recent history, a branch of the Japanese army, Unit 731, reportedly dropped plague-infected fleas in ceramic bomblets over cities in China in WWII, which likely accounts for unusual changes in the epidemiology of this disease in several regions. Prior to the genomic revolution of the last two decades, laboratories in several countries worked with variable success to stabilize infectious microorganisms and toxins so that they could be stored and deployed with greater efficiency and predictability. The advent of molecular biology, advances in our understanding of infectious diseases and immune regulation, and advances in micro-particle engineering and micro-encapsulation have all resulted in technologies that can be used to either advance the properties of biological weapons or as countermeasures to protect against them.

Past military interest in biological weapons was driven by the realization that a comparatively small investment is required to make a tactical weapon capable of killing a large number of enemies. In rare cases, military weapons programs considered biological weapons as part of strategic campaigns. The interest in using biological toxins and infectious microorganisms as weapons was also driven by characteristics of the agents themselves. For example, in contrast with other munitions such as nuclear, chemical and conventional high explosives, only biological weapons are self-replicating. Moreover, these agents can be scaled-up from seed stock to a full stockpile on short notice and with considerably less engineering, manufacturing, capital investment and production signature than would be produced by nuclear or chemical weapons. A related characteristic is that biological weapons can be covertly transported as either minute quantities or in a form that leaves no signature, thus allowing the agents to cross international borders and be produced behind enemy lines. Military strategists also noted that only biological weapons could be successfully deployed without detection, a desirable characteristic if attribution is to be avoided. By the time clinical symptoms would appear, those that deployed the weapon would be many hours or days distant. Most ominously, and in stark contrast to chemical and nuclear weapons, contagious biological weapons such as killer influenza and smallpox, have the unique capacity to cause casualties far beyond the immediate impact zone.

Biological Weapons and Terrorism

Many of the characteristics that make biological weapons attractive to past military programs also make them desirable to the terrorist. Fortunately, the convening of biological weapon capability and terrorist intent has not as yet resulted in a mass-casualty incident. Unfortunately, several disquieting observations of the October 2001 anthrax attack using the U.S. mail system merit emphasis. First, the attack illustrated that advanced expertise had readily been exploited by a bioterrorist; the preparation in the Daschle letter contained extraordinarily high concentrations of purified endospores. Second, the spore preparation was coated with an incipient which helped retard electrostatic attraction, thus increasing aerosolization of the agent. Third, the choice of the near-ubiquitous Ames strain, combined with the absence of forensic details in either the agent or the letters, indicate that the terrorist is scientifically informed, wary of detection and extremely dangerous.

I use this well-publicized case to demonstrate that from the *perspective of the terrorist*, biological weapons are likely to be the optimal choice for inducing terror. As a practical point, the terrorist is likely

to be attracted to any means which causes maximal disruption, terror and loss of confidence while using the minimal amount of skilled personnel, specialized resources and financial investment. For example, the skills required for bioweapon manufacture may be derived from manufacturing practices that use similar technologies such as the fermentative and agricultural sciences, vaccine manufacture, potable water treatment and environmental microbiology. In this regard, bioweapons offer specific advantages for covert manufacture by the terrorist:

1. The agent may be produced using equipment designed for other peaceful purposes (so called 'dual use').
2. Production requires minimal space and time, a characteristic that is increasing with modern technology.
3. Unlike any other weapon, infectious microorganisms are self-perpetuating, and therefore may be propagated among the terrorist groups or cells.
4. Several agents can cause casualties beyond those originally infected.
5. When human assets need to be preserved, these weapons allow the perpetrator to escape detection.

From the perspective of the threat analyst, there are 7 overlapping conditions that need to be present for a terrorist group to produce an effective biological weapon. Failure to meet any of the following conditions can thwart an attempt at weapons production. These conditions are consolidated from consensus opinion of different U.S. Government working groups, by CIMIT's modeling activities and from field experiences working with over one hundred laboratories in Southeast Asia and sub-Saharan Africa (reference Figure 1: a clinical infectious disease laboratory in rural northern Nigeria. The laboratory technician and I are holding up red blood cell agar plates containing the non-hemolytic *Bacillus anthracis* which was isolated from the skin lesion on a local goat herdsman. In this region, estimates of 15-40 cases of cutaneous anthrax are observed annually): the seven conditions for biological weapon production are:

1. Access to agent: this condition requires that the terrorist has the ability to isolate or procure the microorganism or biological toxin. Note that many threat agents are endemic in Neotropical regions of the globe, *including all countries of concern to the U.S.* Naturally-occurring infections resulting from these microorganisms are routinely encountered in domestic animals, *as is the local expertise required to recognize these infections*. Procurement can involve coercion, misrepresentation of intent, or illegal purchase from a former weapons program or strain collection.
2. Reagents: this condition includes availability of factors required for successful biological isolation and amplification. Examples include specialized or improvised culture media, sporulation-inducers, and incipients to stabilize the agent or to improve purity.
3. Expertise: technical know-how can be derived from other disciplines. In modeling studies stated knowledge gaps to weapons manufacture may be overcome using internet based literature and patent reviews, use of out of print texts, and identification of solutions from parallel scientific or manufacturing disciplines.
4. Support technology: this category includes laboratory assets such as roller bottles, agar trays, fermentors, lyophilizers, egg incubators, cold storage capability, animal testing capability and biochemical test kits. The recent commercialization of an unnamed technology has dramatically simplified the challenges to manufacture of one bioweapon by allowing a less refined preparation to be used.

5. Budget: in both resource rich and austere economies, the financial cost of procurement, laboratory consumables, animals and maintenance of laboratory operations is significant. In modeling studies, the anticipated budget required to complete all manufacture tasks posed a greater challenge to a minimally resourced terrorist group than did other tasks.
6. Covert production: modeling for small scale anthrax suggests that a small appropriately-equipped laboratory with a footprint of 250 ft^2 would meet the production needs of a small scale spore weapon. Although many agents can be purified and engineered in simple microbiology laboratories (which are found worldwide), large scale production, coating and stabilization would require a purpose-designated facility.
7. Laboratory Safety: skilled technicians require protection, however the procurement of specialized safety equipment is closely monitored. For this reason safety capability may be improvised, or lab workers may be hyper-vaccinated and maintained on antimicrobial prophylaxis to permit lower levels of containment to be used.

What can the Former Soviet Union Weapons Program teach us about Engineered biological weapons and bioterrorism?

Recent terrorist attacks in Russia have prompted government actions to protect against terrorism. However, an ethnically diverse population, poor border controls, regional corruption, and the continued conflict in Chechnya have all produced conditions that could still result in a biological weapons attack by terrorists. According to one Russian government official, "In no other place do the microbes, the expertise, the infrastructure co-exist in such close proximity with terrorist groups and chaotic times" (name omitted). In the last 2 yrs the concern about terrorism has prompted new levels of disclosure and cooperation between the Russian Federation and the United States. In the last 2 years there have been 4 conferences in Moscow and St Petersburg where prevention and response to bioterrorism was a major topic. These conferences are important for a second reason in that they provide a forum whereby the FSU scientists present previously unknown countermeasures or vaccine strategies which were used to protect production workers or government personnel from the USSR agents. Some recently described technologies, such as non-specific immune enhancers (immune modulators) have little precedence in Western biodefense and are exciting new additions to the BII's Advanced Vaccine and Drug Development program.

Traditional weapons programs

Traditional biological weapon manufacture is best illustrated by the former U.S., British and Soviet era production methods. In the Soviet era program, simple methodologies such as microbial fermentation were conducted on a grander scale. In two former production institutes (Stepnogorsk and Berdsk) fermentors used to produce weapon strains were many thousands of liters in volume, over two stories in height and under continuous stringent environmental control.

In these programs the kill efficiencies of the weapons were increased by maximizing the number of viable microorganisms in the final munition rather than focusing on engineering of the organisms (which came later). SRCAM scientists recount that in the case of anthrax, attention was focused on increasing

fermentation and spore production efficiency, and spore recovery using a number of methods such as foam flotation. Other expertise was directed at improved methods of milling to produce progressively smaller clusters of spores, a condition for successful delivery and sequestration in the terminal alveoli of the lung. By report, there were occasional production misadventures where fermentation runs were contaminated by other bacteria or anti-bacterial phages which destroyed the entire production run.

In the years since the end of the Russian program, our scientific understanding of microbial metabolism and the improved efficiency of automated small scale fermentors have increased the amount of vegetative bacteria that can be produced with minimal resources. Parallel sciences, such as biological insecticides which use bacterial spores afor peaceful purposes, have provided clues to maximize yield in a small laboratory. Perhaps most disturbing is the growing availability of small scale, autonomous operating fermentation systems which reduce the need for skilled technicians and a complex support infrastructure (e.g. Bioflo IV Fermentor, New Brunswick, Inc). These systems are becoming more common in agricultural regions of Africa.

When considered as a whole, traditional weapons technologies with alterations rather than genetic engineering are the most likely to be employed by a moderately resourced, moderately skilled terrorist group. There are many open sources and skilled personnel who can provide guidance to help assemble the critical components necessary for weapons development. Potentially, a former weapons scientist from Stepnogorsk could travel to country in the Middle East and reconvene a weapons capability from available veterinary, agricultural and clinical microbiology resources. For Middle Eastern countries, the easiest solution would be to isolate a virulent epizoonotic pathogen from a local infected animal. These scientists need not bring anything with them but their expertise.

To summarize, efforts to prevent traditional biological weapon production should include efforts to prevent migration of skilled personnel to hostile groups. Additional measures for prevention of weapons development include tight scrutiny of international collaborations and tracking the importation of small scale bacterial growth systems and close human and animal surveillance efforts to detect infections resulting from deficits in the safety of a weapons laboratory.

Next-generation Biological Weapons

Next-generation biological weapons are those that benefit from new technologies, those made from previously unknown infectious agents or biological toxins, and those where a traditional agent is dramatically altered by the addition of a high-tech capability. One concept that is central to discussions of enhanced virulence biological weapons is that the same open source methodologies that advance our ability to improve upon human health may also be commandeered for nefarious purposes. A second point is that traditional biological weapons such as those produced in military weapons programs can be *modernized* to achieve new levels of lethality. The following case is used to illustrate this point.

In the former U.S. weapons program, estimates were made about the number of anthrax spores required for an LD^{50} (dose required to kill 50% of a population) and LD^{90} (dose required to kill 90% of a population). Extrapolations from these estimates indicate that between 8,000-10,000 spores would be required for infection. These estimates are likely accurate for the anthrax strains used in the pre-1971 program. Unfortunately, in recent years there have been dramatic advances in the modeling of airflow in the human lung which in turn has driven the field of aerosolized drug and vaccine delivery. In the last 8

years, particle physicists and pulmonary scientists have worked together to improve the efficiency with which drugs reach the alveoli of the lung, which is also the preferred target for the aerosolized anthrax spore. A parallel advancement has occurred in the field of immunology where new organic coatings have been invented which dramatically increase the uptake of particles by the specialized cells in the alveoli. Unfortunately these cells are also responsible for providing the anthrax bacillus with a protected beachhead for replication. The result is that two unrelated technologies, a method for generating small drug and vaccine aerosols, and the development of a specialized coating, are responsible for dramatically reducing the number of spores required to produce a successful infection. (Figure 3 depicts the methods used to produce a coated anti-floculated spore as well as the calculated reduction in spore concentration required for infecting 80,000 people in a large city. Select steps and information omitted for this testimony)

Genetic engineering has also played a role in altering the capability of biological weapons. Toward the end of the Soviet biological weapons program an effort had been made to make several agents resistant to antibiotics. Much of this work was done using techniques considered inefficient by today's standards. Biological weapon analysts with expertise in molecular biology believe that drug resistant biological weapons are a moderate probability event that could have disastrous consequences. The reasons for this are based in the current health care impact of antibiotic-resistant microorganisms, which are arising as a consequence of indiscriminate antibiotic use. What is not clear is how likely it is that a biological weapons scientist could make a threat agent that is both highly resistant and highly virulent. Such balanced capability would require that the organism be continuously tested against animals to maintain virulence. Thus in this case, the requirements needed to engineer-in genes for antibiotic resistance might also require an attendant investment to insure that the agent remained highly pathogenic.

Next generation biological weapons may also be engineered using *negative selection* techniques. In this case antigens to which the patient's immune response is directed are removed from the biological weapon. In worse case scenarios, the terrorist might eliminate the antigen on a bacteria, virus or toxin that was used as the basis for a government vaccine. If the patient was exposed to one of these antigen-negative biological weapons, they would be immunologically naïve resulting in more severe infection and/or death. These types of agents are known as *vaccine-evading* biological weapons. Unfortunately, the concept that such agents could be developed is dramatically illustrated by the need for new vaccines to protect against circulating strains of influenza A/H3N2.

Next-generation biological weapons also include the engineering-in of properties that influence the ability of the body to mount an immune response. In recent years, there have been several publications which have demonstrated this concept to biodefense scientists and potentially, to any terrorist with internet access. One of the most disquieting publication in 2002 described a method for defeating vaccine-protected animals by inserting a gene which down-regulated the immune system resulting in overwhelming infection and depth (reference provided upon request). Another publication which will appear in an international journal this September describes a methodology which single-handedly solves two separate challenges facing a biological terrorist: how to move virulence genes from one agent to another, and how to store a biological weapon without depending on freezers and liquid nitrogen (reference provided upon request).

One of the most ominous of engineering feats that could be used by biological weapon scientists is to induce host tropism into the agent, whereby the agent is altered to favor infection of a specific human

genotype. This seemingly far-fetched concept is already demonstrated by certain tropical parasite infections that cause more significant infections and sequelae in certain ethnic groups.

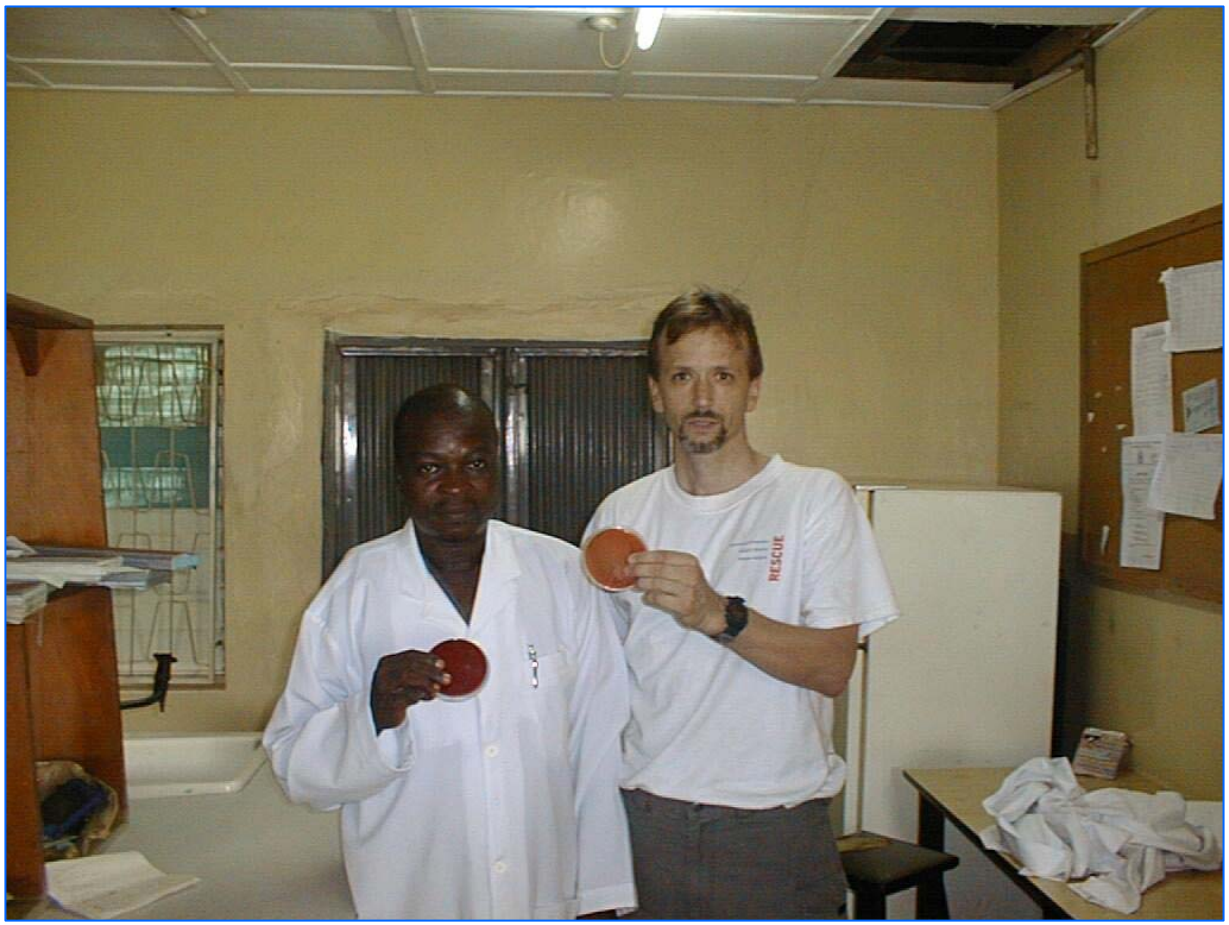
The efforts of the biological terrorist to produce a new threat agent can also be assisted by natural events. This scenario is best illustrated by current experience with avian influenza in Southeast Asia. Since 1998, the pathogenicity of this bird virus has increased as has its ability to infect the upper respiratory systems of pigs and humans. The result is that infected patients are exposed to a novel, highly pathogenic respiratory virus to which their immune system is completely naive. The danger of this event is exacerbated by the fact that influenza, unlike anthrax, can be transmitted from person to person.

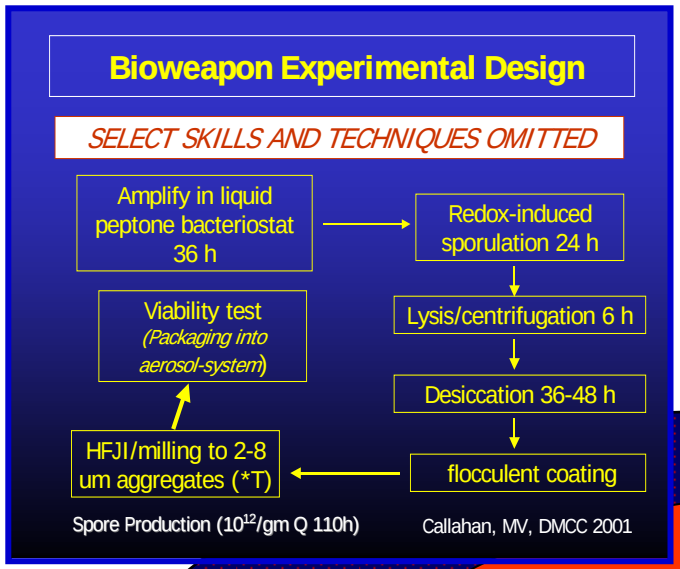
I will summarize this written testimony by reaffirming the concept that the dark science of biological weapon design and manufacture parallels that of the health sciences and the cross mixed disciplines of modern technology. Potential advances in biological weapon lethality will in part be the byproduct of peaceful scientific progress. So, until the time when there are no more terrorists, the U.S. Government and the American people will depend on the scientific leaders of their field to identify any potential dark side aspect to every achievement

Again, I appreciate the opportunity to present this information before the Committee. I shall be happy to answer your questions and to provide additional documentation supporting the material presented.

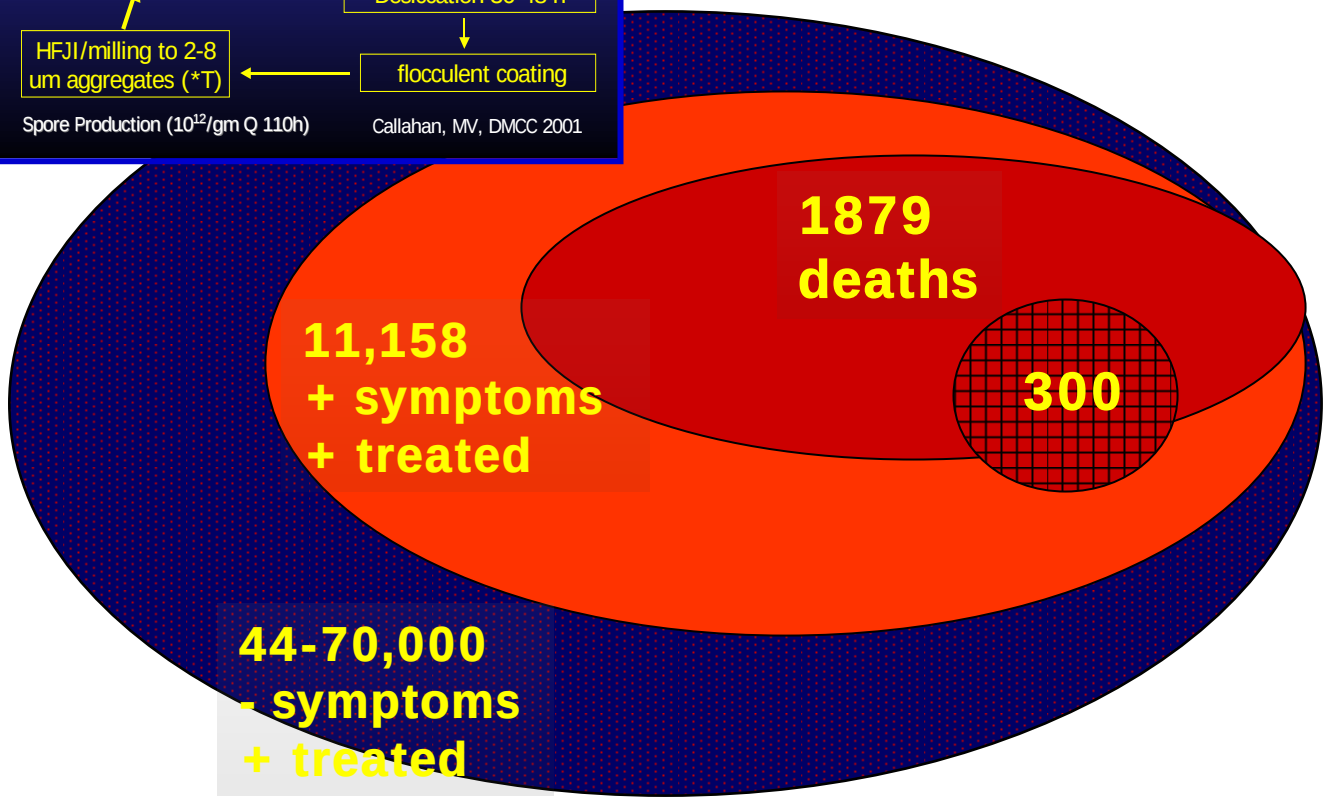
Figure 1: (LEFT) Smallpox collection at VECTOR's building 6B, Kotsovo, Russia.Vector and the CDC are the only 2 centers publicly acknowledged as having smallpox (RIGHT) BL4 Russian P3 containment in Building 1

Figure 2: Anthrax bacillus growing on improvised red blood cell agar. The pathogen was recovered from the skin lesion of a rural goat rancher. Clinical microbiology laboratory in Kaduna Region, Nigeria





Production scheme and morbidity-mortality footprint from point source open-air mXXXd-pXXX hardened spore aerosol in city (2200/km^2)



= acute fatalities

WT (2k MOI)

No Detection

Point source

2200 hs/km^2

Callahan et al.
NBACC Jan 2004

Figure 3